(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification[7]: B01L 3/00

(21) International Application Number: PCT/EP00/12478

(22) International Filing Date:
11 December 2000 (11.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9904802-7   23 December 1999 (23.12.1999)   SE

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— With international search report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: MICROFLUIDIC SURFACES

(57) Abstract: A microfluidic device comprising a set of one or more, preferably more than 5, covered microchannel structures manufactured in the surface of a planar substrate. The device is characterized in that a part surface of at least one of the microchannel structures has a coat exposing a non-ionic hydrophilic polymer. The non-ionic hydrophilic polymer is preferably attached covalently directly to the part surface or to a polymer skeleton that is attached to the surface.

MICROFLUIDIC SURFACES

Technical field

5 The invention concerns a microfluidic device comprising a set
of one or more, preferably more than 5, covered microchannel
structures fabricated in the surface of a planar substrate.

By the term "covered" is meant that a lid covers the
10 microchannel structures thereby minimising or preventing
undesired evaporation of liquids. The cover/lid may have
microstructures matching each microchannel structure in the
substrate surface.

15 The term "fabricated" means that two-dimensional and/or three-
dimensional microstructures are present in the surface. The
difference between a two-dimensional and a three-dimensional
microstructure is that in the former variant there are no
physical barriers delineating the structure while in the
20 latter variant there are. See for instance WO 9958245 (Larsson
et al).

The part of the cover/lid, which is facing the interior of a
microchannel is included in the surface of a microchannel
25 structure.

The planar substrate typically is made of inorganic and/or
organic material, preferably of plastics. For examples of
various inorganic and organic materials see under the heading
30 "Material in the microfluidic device".

A microfluidic device encompasses that there is a liquid flow
that causes mass transport of solutes and/or particles
dispersed in the liquid from one functional part of the

structure to another. Sole capillaries, possibly with an area
for application and an area for detection, as used in
capillary electrophoresis in which solutes are caused to
migrate by an applied electric field for separation purposes
5 are not microfluidic devices as contemplated in the context of
the invention. An electrophoresis capillary may, however, be
part of a microfluidic device if the capillary is part of a
microchannel structure in which there are one or more
additional functional parts from and/or to which mass
10 transport of a solute by a liquid flow is taking place as
defined above.

The liquid is typically polar, for instance aqueous such as
water.
15

Technical background.
Microfluidic devices require that liquid flow easily pass
through the channels and that non-specific adsorption of
reagents and analytes should be as low as possible, i.e.
20 insignificant for the reactions to be carried out.

Reagents and/or analytes includes proteins, nucleic acids,
carbohydrates, cells, cell particles, bacteria, viruses etc.
Proteins include any compound exhibiting poly- or oligopeptide
25 structure.

The hydrophilicity of surfaces within microchannel structures
shall support reproducible and predetermined penetration of an
aqueous liquid into the various parts of a structure. It is
30 desirable that once the liquid has passed a possible break at
the entrance of a part of the structure then the liquid
spontaneously shall enter the part by capillary action
(passive movement). This in turn means that the hydrophilicity
of the surfaces within microchannel structures becomes of

increasing importance when going from a macroformat to a microformat.

From our experience, water contact angles around 20 degrees or
5 lower may often be needed to accomplish reliable passive fluid
movement into microchannel structures. However, it is not
simple to manufacture surfaces which permanently have such low
water contact angles. There is often a tendency for a change
in water contact angles during storage, which renders it
10 difficult to market microfluidic devices having standardised
flow properties.

The situation is complicated by the fact that methods for
preparing surfaces with very low water contact angles do not
15 necessarily reduce the ability to non-specifically adsorb
reagents and sample constituents. The surface/volume ratio
increases when going from a macroformat down to smaller
formats. This means that the capacity for non-specific
adsorption of a surface increases inversely with the volume
20 surrounded by the surface. Non-specific adsorption therefore
becomes more critical in microformat devices than in larger
devices.

An unacceptable non-specific adsorption of biomolecules is
25 often associated with the presence of hydrophobic surface
structures. This particular problem therefore is often more
severe in relation to surfaces made of plastics and other
hydrophobic materials compared to surfaces of native silicon
surfaces and other similar inorganic materials.
30
There are a number of methods available for treating surfaces
to make them hydrophilic in order to reduce non-specific
adsorption of various kinds of biomolecules and other
reagents. However, these methods generally do not concern
35 balancing a low non-specific adsorption with a reliable and

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reproducible liquid flow when miniaturizing macroformats down
into microformats. Compare for instance Elbert et al., (Annu.
Rev. Mater. Sci. 26 (1996) 365-394).

5 Surfaces that have been rendered repelling for biopolymers in
general by coating with adducts between polyethylenimines and
hydrophilic polymers have been described during the last
decade (Brink et al (US 5,240,994), Bergström et al., US
5,250,613; Holmberg et al., J. Adhesion Sci. Technol. 7(6)
10 (1993) 503-517; Bergström et al., Polymer Biomaterials, Eds
Cooper, Bamfors, Tsuruta, VSP (1995) 195-204; Holmberg et al.,
Mittal Festschrift, Eds Van Ooij, Anderson, VSP 1998, p 443-
460; and Holmberg et al., Biopolymers at Interfaces, Dekker
1998 (Surfactant Science Series 75), 597-626). Sequential
15 attachment of a polyethylenimine and a hydrophilic polymer has
also been described (Kiss et al., Prog. Colloid Polym. Sci. 74
(1987) 113-119).

Non-specific adsorption and/or electroendosmosis have been
20 controlled in capillary electrophoresis by coating the inner
surface of the capillary used with a hydrophilic layer,
typically in form of a hydrophilic polymer (e.g. van Alstine
et al US 4,690,749; Ekström & Arvidsson WO 9800709; Hjertén,
US 4,680,201 (poly methacrylamide); Karger et al., US
25 5,840,388 (polyvinyl alcohol (PVA)); and Soane et al., US
5,858,188 and US 6,054,034 (acrylic microchannels). Capillary
electrophoresis is a common name for separation techniques
carried out in a narrow capillary utilizing an applied
electric filed for mass transport and separation of the
30 analytes.

Larsson et al (WO 9958245, Amersham Pharmacia Biotech)
presents among others a microfluidic device in which
microchannels between two planar substrates are defined by the
35 interface between hydrophilic and hydrophobic areas in at

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least one of the substrates. For aqueous liquids the
hydrophilic areas define the fluid pathways. Various ways of
obtaining a pattern of hydrophobic and hydrophilic surfaces
for different purposes are discussed, for instance, plasma

5 treatment, coating a hydrophobic surfaces with a hydrophilic
polymer etc. The hydrophilic coat polymers suggested may or
may not have aryl groups suggesting that Larsson et al are not
focusing on lowering the water contact angle as much as
possible or avoiding non-specific adsorption.

10

Larsson, Ocklind and Derand (PCT/EP00/05193 claiming priority
from SE 9901100-9, filed 1999-03-24) describe the production
of highly hydrophilic surfaces made of plastics. The surfaces
retain their hydrophilicity even after being in contact with

15 aqueous liquids. An additional issue in PCT/EP00/05193 is to
balance a permanent hydrophilicity with good cell attachment
properties. The surfaces are primarily suggested to be used in
microfabricated devices.

20 Polyethylene glycol has been linked directly to the surface of
a microchannel fabricated in silicone for testing the ability
of polyethylyne glycol to prevent protein adsorption. See
Bell, Brody and Yager (SPIE-Int. Soc. Opt. Eng. (1998) 3258
(Micro- and Nanofabricated Structures and Devices for

25 Biomedical Environmental Applications) 134-140).

The objectives of the invention.
A first objective is to accomplish a sufficiently reliable and
reproducible mass transport of reagents and sample

30 constituents (e.g. analytes) in microfluidic devices.

A second objective is to enable a reliable and reproducible
aqueous liquid flow in the microfluidic devices.

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A third objective is to optimise non-specific adsorption and
hydrophilicity in relation to each other for surfaces of fluid
pathways in microfluidic devices.

5

The invention

We have discovered that by attaching a hydrophilic non-ionic
polymer to the surface of a microchannel structure in a
microfluidic device one can easily minimize the above-

10 mentioned problems also for the most critical surface
materials. This discovery facilitates creation of surfaces
that permit reliable and reproducible transport of reagents
and sample constituents in microfluidic devices.

15 The main aspect of the invention is a microfluidic device as
defined under the heading "Technical Field". The characterizing
feature is that at least a part surface of each microchannel
structure exposes a firmly attached non-ionic hydrophilic
polymer to the interior of the structure.

20

The non-ionic hydrophilic polymer may be attached directly to
the surface of the microchannel structure or via a polymer
skeleton that in turn is attached to the surface via
multipoint attachment.

25 **The non-ionic hydrophilic polymer**

The non-ionic hydrophilic polymer contains a plurality of
hydrophilic neutral groups. Neutral groups excludes non-
charged groups that can be charged by a pH-change. Typical
neutral hydrophilic groups contains an heteroatom (oxygen,

30 sulphur or nitrogen) and may be selected among hydroxy, ether
such as ethylene oxy (e.g. in polyethylene oxide), amides that
may be N-substituted etc. The polymer as such is also inert
towards the reagents and chemicals that are to be used in the
microfluidic device.

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Illustrative non-ionic hydrophilic polymers are preferably water-soluble when not bound to a surface. Their molecular weight is within the range from about 400 to about 1,000,000 daltons, preferably from about 1,000 to about 2000,000, such
5 as below 100,000 daltons.

Non-ionic hydrophilic polymers are illustrated with polyethylene glycol, or more or less randomly distributed or block-distributed homo- and copolymers of lower alkylene
10 oxides (C_{1-10}, such as C_{2-10}) or lower alkylene (C_{1-10}, such as C_{2-10}) bisepoxides in which the epoxide groups are linked together via a carbon chain comprising 2-10 sp^3-carbons. The carbon chain may be interrupted at one or more positions by an ether oxygen, i.e. an ether oxygen is inserted between two carbon
15 atoms. A hydrogen atom at one or more of the methylene groups may be replaced with hydroxy groups or lower alkoxy groups (C_{1-4}). For stability reasons at most one oxygen atom should be bound to one and the same carbon atom.

20 Other suitable non-ionic hydrophilic polymers are polyhydroxy polymers that may be completely or partly natural or completely synthetic.

Completely or partly natural polyhydroxy polymers are
25 represented by polysaccharides, such as dextran and its water-soluble derivatives, water-soluble derivatives of starch, and water-soluble derivatives of cellulose, such as certain cellulose ethers. Potentially interesting cellulose ethers are methyl cellulose, methyl hydroxy propyl cellulose, and ethyl
30 hydroxy ethyl cellulose.

Synthetic polyhydroxy polymers of interest are also polyvinyl alcohol possibly in partly acetylated form, poly(hydroxy lower alkyl vinyl ether) polymers, polymers obtained by

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polymerisation of epichlorohydrin, glycidol and similar
bifunctionally reactive monomers giving polyhydroxy polymers.

Polyvinylpyrrolidone (PVP), polyacrylamides,
5 polymethacrylamides etc are examples of polymers in which
there are a plurality of amide groups.

Further suitable hydrophilic polymers are reaction products
(adducts) between ethylene oxide, optionally in combination
10 with higher alkylene oxides or bisepoxides, or
tetrahydrofuran, and a dihydroxy or polyhydroxy compound as
illustrated with glycerol, pentaerythritol and any of the
polyhydroxy polymers referred to in the preceding paragraphs.

15 The non-ionic hydrophilic polymer may have the same structure
as described for the extenders defined in Berg et al (WO
9833572) which is hereby incorporated by reference. In
contrast to Berg et al there is no imperative need for the
presence of an affinity ligand on the hydrophilic polymer used
20 in the present invention.

One or more positions in the non-ionic hydrophilic polymer may
be utilized for attachment. In order to make the hydrophilic
polymer flexible the number of attachment points should be as
25 low as possible, for instance one, two or three positions per
polymer molecule. For straight chain polymers, such as lower
alkylene oxide polymers similar to polyethylene oxide, the
number of attachment points is typically one or two, with
preference for one.
30

Depending on the position of a coated part surface within a
microchannel structure, the hydrophilic polymer may carry an
immobilized reactant (often called ligand when affinity
reactions are concerned). Depending on the particular use of a
35 microchannel structure such reactants can be so called

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affinity reactants that are used to catch an analyte or an
added reactant or a contaminant present in the sample.
Immobilized ligands also include immobilized enzymes.
According to the invention this kind of reactants are
5 preferably present in reaction chambers/cavities (see below).

The skeleton
The skeleton may be an organic or inorganic cationic, anionic
or neutral polymer of inorganic or organic material.
10

With respect to inorganic skeletons, the preferred variants
are polymers such as silicon oxide. See the experimental part.

With respect to organic skeletons, the preferred variants are
15 cationic polymers, such as a polyamine, i.e. a polymer
containing two or more primary, secondary or tertiary amine
groups or quaternary ammonium groups. The preferred polyamines
are polyalkylenimines, i.e. polymers in which amine groups are
interlinked by alkylene chains. The alkylene chains are for
20 instance selected among C_{1-6} alkylene chains. The alkylene
chains may carry neutral hydrophilic groups, for instance
hydroxy (HO) or poly (including oligo) lower alkylene oxy
groups $[-O-((C_2H_4)_nO)_mH$ where n is 1-5 and m is from 1 and
upwards for instance ≤ 100 or $\leq 50)$], amide groups, acyl,
25 acyloxy, lower alkyl (for instance C_{1-5}) and other neutral
groups and/or groups that are unreactive under the conditions
to be applied in the microfluidic device.

The preferred molecular weight of the skeleton including
30 polyamine skeletons is within the range of 10,000-3,000,000
daltons, preferably about 50,000-2,000,000 daltons. The
structure of the skeleton can be linear, branched,
hyperbranched or dendritic. The preferred polyamine skeleton
is polyethylenimine, a compound that is achievable e.g. by

polymerizing ethylene imine, usually giving hyperbranched
chains.

Attachment of the non-ionic hydrophilic polymer

5 The introduction of the non-ionic hydrophilic polymer groups
on the channel surfaces may be done according to principles
well-known in the field, forinstance by directly attaching the
hydrophilic polymer to the desired part surface or via the
kind of skeleton discussed above. The adduct between the
10 skeleton and the non-ionic hydrophilic polymer may be (i)
formed separately before it is attached to the surface or (ii)
on the surface by first attaching the skeleton and then the
hydrophilic polymer. Alternative (ii) can be carried out by
(a) grafting a preprepared non-ionic hydrophilic polymer to
15 the skeleton or (b) graft polymerisation of suitable monomers.

Both the non-ionic hydrophilic polymer and the skeleton may be
stabilized to the underlying surfaces via covalent bonds,
electrostatic interaction etc and/or by cross-linking *in situ*
20 or afterwards. A polyamine skeleton, for instance, may be
attached covalently by reacting its amine functions with
aminereactive groups that are originally present or have been
introduced on the uncoated substrate surface.
It is important that the nude part surface to be coated
25 according to the invention has groups, which enable stable
interaction between the non-ionic hydrophilic polymer and the
surface and between the skeleton and the surface. Cationic
skeletons, for instance polyamines, require that negatively
charged or chargeable groups or groups otherwise capable of
30 binding to amine groups, typically hydrophilic, are exposed on
the surface. Polar and/or charged or chargeable groups may
easily be introduced on plastics surfaces, for instance by
treatment with O_2- and acrylic acid-containing plasmas, by
oxidation with permanaganate or bichromate in concentrated
35 sulphuric acid, by coating with polymers containing these type

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of groups etc. In other words by techniques well-known in the scientific and patent literature. The plastics surface as such may also contain this kind of groups without any pretreatment, i.e. by being obtained from polymerisation of monomers either
5 carrying the above-mentioned type of groups or groups that subsequent to polymerisation easily can be transformed to such groups.

If the surface to be coated is made of a metal, for instance
10 of gold or platina, and the non-ionic hydrophilic polymer or skeleton has thiol groups, attachment can be accomplished via bonds that are partly covalent.

If the non-ionic hydrophilic polymer or the skeleton have
15 hydrocarbon groups, for instance pure alkyl groups or phenyl groups, one can envisage that attachment to the substrate surface can take place via hydrophobic interactions.

Water contact angles
20 The optimal water contact angle depends on the analyses and reactions to be carried out in the microchannel structure, dimensions of the microchannels and chambers of the structures, composition and surface tension of liquids used, etc. As a rule of thumb, the inventive coat should be selected
25 to provide a water contact angle that is ≤ 30°, such as ≤ 25° or ≤ 20°. These figures refer to values obtained at the temperature of use, primarily room temperature.

So far the most superior surfaces have been those based on
30 adducts between polyethylene imine and polyethylene glycol with monosite (mono group terminal) attachment of the non-ionic hydrophilic polymer to the polyethylene imine skeleton. The best mode to date of this preferred variant is given in the experimental part (example 1).

Thickness of the coat

The thickness of the hydrated coat provided by the non-ionic
hydrophilic polymers should be ≤ 50 %, for instance ≤ 20 % of
5 the smallest distance between two opposing sides of a part of
the microchannel structure comprising the surface coated
according to the invention. This typically means that an
optimal thickness will be within the interval 0.1-1000 nm, for
instance 1-100 nm, with the provision that the coat shall
10 permit a desired flow to pass through.

Structures in the microfluidic device.

The microfluidic device may be disc-formed of various
geometries, with the round form being the preferred variant
15 (CD-form).

On devices having round forms, the microchannel structures may
be arranged radially with an intended flow direction from an
inner application area radially towards the periphery of the
20 disc. In this variant the most practical ways of driving the
flow is by capillary action, centripetal force (spinning the
disc) and/or hydrodynamically.

Each microchannel structure comprises one or more channels
25 and/or one or more cavities in the microformat. Different
parts of a structure may have different discrete functions.
Thus there may be one or more parts that function as (a)
application chamber/cavity/area (b) conduit for liquid
transport, (c) reaction chamber/cavity, (d) volume defining
30 unit, (e) mixing chamber/cavity, (f) chamber for separating
components in the sample, for instance by capillary
electrophoresis, chromatography and the like (g) detection
chamber/cavity, (h) waste conduit/chamber/cavity etc.
According to the invention at least one of these parts may

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have the inventive coat on its surface, i.e. corresponds to
the part surface discussed above.

When the structure is used, necessary reagents and/or sample
5 including the analyte are applied to an application area and
transported downstream in the structure by an applied liquid
flow. Some of the reagents may have been predispensed to a
chamber/cavity. The liquid flow may be driven by capillary
forces, and/or centripetal force, pressure differences applied
10 externally over a microchannel structure and also other non-
electrokinetic forces that are externally applied and cause
transport of the liquid and the analytes and reagents in the
same direction. The liquid flow may also be driven by pressure
generated by electroendoosmosis created within the structure.
15 The liquid flow will thus transport reagents and analytes and
other constituents from an application area/cavity/chamber
into a sequence comprising a particular order of preselected
parts (b)-(h). The liquid flow may be paused when a reagent
and/or analyte have reached a preselected part in which they
20 are subjected to a certain procedure, for instance capillary
electrophoresis in a separation part, a reaction in a reaction
part, detection in a detection part etc.

Analytical and preparative methods as discussed below
25 utilizing the microfluidic device of the invention with
transport of liquid, reagents and analytes as described in the
preceding paragraph constitute a separate aspect of the
invention.

30 Microformat means that at least one liquid conduit in the
structure has a depth and/or width that is in the microformat
range, i.e. $< 10^3$ µm, preferably $< 10^2$ µm. Each microchannel
structure extends in a common plane of the planar substrate
material. In addition there may be extensions in other

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directions, primarily perpendicular to the common plane. Such
other extensions may function as sample or liquid application
areas or connections to other microchannel structures that are
not located in the common plane, for instance.

5

The distance between two opposite walls in a channel is ≤ 1000
μm, such as ≤ 100 μm, or even ≤ 10 μm, such as ≤ 1 μm. The
structures may also contain one or more chambers or cavities
connected to the channels and having volumes being ≤ 500 μl,
10 such as ≤ 100 μl and even ≤ 10 μl such as ≤ 1 μl. The depths of
the chambers/cavities may typically be in the interval ≤ 1000
μm such as ≤ 100 μm such as ≤ 10 μm or even ≤ 1 μm. The lower
limit is always significantly greater than the largest of the
reagents used. The lower limits of chambers and channels are
15 typically in the range 0.1-0.01 μm for devices that are to be
delivered in dry form.

It is believed that the preferred variants of the inventive
microfluidic devices will be delivered to the customer in a
20 dried state. The surfaces of the microchannel structures of
the device therefore should have a hydrophilicity sufficient
to permit the aqueous liquid to be used to penetrate the
different parts of the channels of the structure by capillary
forces (self-suction).

25

There may be conduits enabling liquid communication between
individual microchannel structures within a set.

Material in the microfluidic device.
30 The surface to be coated according to the invention typically
is made of inorganic and/or organic material, preferably of
plastics. Diamond material and other forms of elemental carbon
are included in the term organic material. Among suitable

inorganic surface materials can be mentioned metal surfaces,
e.g. made of gold, platina etc.

Plastics to be coated according to the invention may have been
5 obtained by polymerisation of monomers comprising unsaturation
such as carbon-carbon double bonds and/or carbon-carbon-triple
bonds.

The monomers may, for instance, be selected from mono-, di and
10 poly/oligo-unsaturated compounds, e.g. vinyl compounds and
other compounds containing unsaturation. Illustrative monomers
are:

(i)   alkenes/alkadienes (such as ethylene, butadiene,
        propylene and including substituted forms such as vinyl
15   ethers), cycloalkenes, polyfluorovinyl hydrocarbons
        (for instance tetrafluoroethylene), alkene-containing
        acids, esters, amides, nitriles etc for instance
        various methacryl/acryl compounds; and

(ii)   vinyl aryl compounds (such as mono-, di- and trivinyl
20   benzenes) that optionally may be substituted with for
        instance lower alkyl groups (C1-6) etc.

Another type of plastics are based on condensation polymers in
which the monomers are selected from compounds exhibiting two
25 or more groups selected among amino, hydroxy, carboxy etc
groups. Particularly emphasised monomers are polyamino
monomers, polycarboxy monomers (including corresponding
reactive halides, esters and anhydrides), poly hydroxy
monomers, amino-carboxy monomers, amino-hydroxy monomers and
30 hydroxy-carboxy monomers, in which poly stands for two, three
or more functional groups. Polyfunctional compounds include
compounds having a functional group that is reactive twice,
for instance carbonic acid or formaldehyde. The plastics
contemplated are typically polycarbonates, polyamides,

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polyamines, polyethers etc. Polyethers include the
corresponding silicon analogues, such as silicone rubber.

The polymers of the plastics may be in cross-linked form.

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The plastics may be a mixture of two or more different
polymer(s)/copolymer(s).

Particularly interesting plastics are those that have a non-
10 significant fluorescence for excitation wavelengths in the
interval 200-800 nm and emission wavelengths in the interval
400-900 nm. By non-significant fluorescence is meant that the
fluorescence intensity in the above-given emission wavelength
interval should be below 50 % of the fluorescence intensity
15 for a reference plastics (= a polycarbonate of bisphenol A
without fluorescent additives). In fact it does not harm in
case the fluorescence intensity of the plastics is even lower,
such as < 30 % or < 15 %, such as < 5 % or < 1 %, of the
fluorescence intensity of the reference plastics. Typical
20 plastics having an acceptable fluorescence are based on
polymers of aliphatic monomers containing polymerizable
carbon-carbon double bonds, such as polymers of cykloalkenes
(e.g. norbornene och substituted norbornenes), ethylene,
propylenes etc, as well as other non-aromatic polymers of high
25 purity, e.g. certain grades of polymethylmethacrylate.

In preferred variants of the invention the same limits for
fluorescence also apply to the microfluidic structure after
having been coated in accordance with the invention.

30

**Applications in which the inventive microfluidic device can be
used.**
The primary use of the microfluidic devices of the invention
is in analytical and preparative chemical and biochemical
35 systems.

Typical analytical systems in which the microfluidic systems
described herein may comprise as the main steps one or more of
(a) sample preparation, (b) assay reactions and (c) detection.
5 Sample preparation means the preparation of a sample in order
to make it suitable for the assay reactions and/or for the
detection of a certain activity or molecular entity. This may
for example mean that substances interfering with the assay
reactions and/or detection is removed or otherwise
10 neutralized, that substances are amplified and/or derivatized
etc. Typical examples are (1) amplifying one or more nucleic
acid sequences in a sample, for instance by polymerase chain
reaction (PCR), (2) removing of species cross-reacting with an
analyte in assays involving affinity reactions etc. Typical
15 assay reactions are (i) reactions involving cells, (ii)
affinity reactions, for instance biospecific affinity
including immune reactions, enzymatic reactions,
hybridization/annealing etc, (iii) precipitation reactions,
(iv) pure chemical reactions involving formation or breaking
20 up of covalent bonds, etc. The detection reaction may involve
fluorometry, chemiluminometry, mass spectrometry,
nephelometry, turbidometry etc. The detection reaction aims at
detection of the result of the assay reaction(s) and at
relating a found result with the qualitative or quantitative
25 presence of an activity in the original sample. The activity
can be a biological, a chemical, a biochemical etc activity.
It may be as the presence of a compound as such or simply as
an activity of a known or unknown compound. If the system is
used for diagnostic purposes the result in the detection step
30 is further correlated to the medicinal status of the
individual from which the sample derives. The applicable
analytical systems may thus comprise affinity assays, such as
immune assays, hybridisation assays, cell biology assays,
mutation detection, genome characterisation, enzyme assays,
35 screening assays for finding new affinity pairs etc. Methods

for the analysis of sample content of proteins, nucleic acids, carbohydrates, lipids and other molecules with particular emphasis of other bio-organic molecules are also included.

5 The microfluidic device of the present invention may also find use for the set up of libraries of compounds including synthetic peptide and oligonucleotide libraries, for instance by solid phase synthesis. The synthesis of so called combinatorial libraries of compounds is also included.

10

The invention will now be described with reference to non-limitative experiments that function as proof of principle.

EXPERIMENTAL PART

15

A. COAT OF PEG-PEI ADDUCT

a. Synthesis of PEG-PEI adduct

0.43 g of polyethylenimine (Polymin SN from BASF, Germany) was
20 dissolved in 45 ml of 50 mM sodium borate buffer (pH 9.5) at 45°C. 5 g of the glycidyl ether of monomethoxy polyethylene glycol (Mw 5 000) was added during stirring and the mixture was stirred for 3 h at 45°C.

25 b. Surface treatment

A polycarbonate CD disc (polycarbonate of Bisphenol A, Macrolon DP-1265, Bayer AG, Germany) with a recessed microchannel pattern was placed in a plasma reactor (Plasma Science PS0500, BOC Coating Technology, USA) and treated with
30 an oxygen plasma at 5 sccm gas flow and 500 W RF power for 10 min. After venting the reactor, the disc was immersed in a 0.1% solution of the PEG-PEI adduct in borate buffer pH 9.5 for 1 h. The disc was then rinsed with distilled water, blown dry with nitrogen and the water contact angle (sessile drop)
35 was measured on a Ramé-Hart manual goniometer bench. The average of six equilibrium measurements (three droplets) was

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24 degrees. An XPS spectrum of the treated surface gave the
following molar elemental composition: 73.2% C, 3.7 % N, 23.1%
O, showing that the surface was essentially covered by the
adsorbed PEG-PEI adduct.

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c. Capillary wetting

Another polycarbonate CD disc of the same material as above
with a recessed microchannel pattern was treated as in example
2. It was then covered with a thin silicone rubber lid, with a
10 hole placed over a microchannel. When a droplet of water was
placed in the hole with a micropipette, the water was drawn in
by capillary forces and penetrated the entire accessible
channel system.

15 **d. Comparative examples of surface treatments**
a) A polycarbonate disc of the same material as above with a
   recessed microchannel pattern was dipped into a 0.5% water
   solution of phenyl dextran (degree of substitution: 0.2 per
   monosacharide unit of dextran, Mw 40 000) for 1 h. After
20   water rinsing, the disc was blown dry with nitrogen. The
   water contact angle was 30 degrees. When a silicone rubber
   lid was placed over the disc with a hole over a channel, the
   droplet was not spontaneously drawn in. When a vacuum was
   applied to the channel through another hole in the lid, the
25   droplet could however be introduced by suction.
b) A polycarbonate disc of the same material as above with a
   recessed microchannel pattern was immersed over night in a 1
   % water solution of a polyethylene glycol "polypropylene
   glycol" polyethylene glycol triblock copolymer (Pluronic
30   F108 from BASF). After water rinsing the disc was blown dry
   with nitrogen. The water contact angle was 60 degrees. When
   a silicone rubber lid was placed over the disc with a hole
   over a channel, the droplet was not spontaneously drawn in.
   When a vacuum was applied to the channel through another

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hole in the lid, the droplet could however be introduced by suction.

B. POLY(ACRYLAMIDE) COATING.

a) Activation of the surface.

A PET foil (polyethylene terephthalate, Melinex®, ICI), evaporation coated with a thin film of silicon oxide, was used as a lid. The silicon oxide side of the PET foil was washed with ethanol and thereafter UV/Ozone (UVO cleaner, Model no 144A X-220, Jelight Company, USA) treated for 5 minutes. 15 mm Bind silane (3-methacryloloxypropyl trimethoxysilane, Amersham Pharmacia Biotech), 1.25 ml 10% acetic acid and 5 ml ethanol was mixed and thereafter applied onto the foil using a brush. After evaporation of the solvent, the foil was washed with ethanol and blown dry with nitrogen. The water contact angle (sessile drop) was measured on a Ramé-Hart manual goniometer. The average of repeated measurements was 62 degrees.

b. Grafting polyacrylamide to the activated surface

8.5 ml of 3 M acrylamide in water and 1.5 ml of 100 mM Irgacure 184 (dissolved in ethylene glycol, Ciba-Geigy) was mixed. The resulting solution was spread out on a quartz plate, and the activated PET foil was placed on top. The monomer solution was UV illuminated for 20 minutes through the quartz plate. The PET foil was then washed thoroughly in water and the average contact angle of repeated measurements was 17 degrees.

c. Capillary wetting

A piece of room temperature vulcanizing silicone rubber (Memosil, Wacker Chemie) having a microchannel structure and two holes was placed onto the polyacrylamide grafted PET foil (lid) (according to b above). When a droplet of water was

placed in the hole with a micropipette, the water was drawn in
by capillary forces.

d. Comparative example of capillary wetting

5 A piece of room temperature vulcanizing silicone rubber
(Memosil, Wacker Chemie) having a microchannel pattern and two
holes were placed onto the activated PET foil (lid) (according
to a above). When a droplet of water was placed in the hole
with a micropipette, no water was drawn in by capillary
10 forces. When vacuum was applied to the channel through the
other hole, the droplet was sucked into the channel.

22
C L A I M S

1. A microfluidic device comprising a set of one or more,
   preferably more than 5, covered microchannel structures
   manufactured in the surface of a planar substrate,
5 **characterized** in that a part surface of at least one of the
   microchannel structures has a coat exposing a non-ionic
   hydrophilic polymer that preferably is attached covalently
   directly to the surface or to a polymer skeleton that is
   attached to the surface.
10

2. The microfluidic device of claim 1, **characterized** in that
   the surface of the planar substrate is made of plastics.

3. The microfluidic device according to any of claims 1-2,
15 **characterized** in that the non-ionic hydrophilic polymer is
   attached to the polymer skeleton that is attached to the
   part surface, said skeleton preferably being branched and/or
   preferably being a polyamine.

20 4. The microfluidic device according to any of claims 1-3,
   characterized in that the substrate surface without the coat
   is made of plastics and that said part surface without coat
   is hydrophilized by plasma treatment or by an oxidation
   agent in order to introduce functional groups that allow for
25   a subsequent attachment of the coat onto said part surface.

5. The microfluidic device according to any of claims 1-4,
   characterized in that the non-ionic hydrophilic polymer
   comprises one or more blocks of polyoxyethylene chains, with
30   preference for the polymer being polyethylene glycol
   covalently attached at one of its ends to the skeleton or
   directly to the part surface and possibly having the
   remaining hydroxy group etherified.

6. The microfluidic device according to any of claims 1-6, **characterized** in that the hydrophilic non-ionic polymer is a polyethylene glycol, preferably a monoalkoxy variant such as the monomethoxy variant, which is attached to said part

5   surface via the polymer skeleton which preferably is a polyethylenimine.

7. The microfluidic device according to any of claims 1-6, **characterized** in that the hydrophilic non-ionic polymer is

10   attached to said part surface or to said polymer skeleton via one-point attachment, preferably covalently.

8. The microfluidic device according to any of claims 2-7, **characterized** in that the plastics has a non-significant

15   fluorescence for excitation wavelengths in the interval 200-800 nm and emission wavelengths in the interval 400-900 nm.

9. The microfluidic device according to any of claims 1-3 and 5-8, **characterized** in that said polymer skeleton is an

20   inorganic or an organic polymer.

10.   The microfluidic device according to any of claims 1-4 and 7-9, **characterized** in that said non-ionic hydrophilic polymer comprises a plurality of amide bonds, e.g. is

25   polymerisate/copolymerisate with monomers at least selected from acrylamide, methacrylamide, vinylpyrrolidone etc.

11.   The microfluidic device according to any of claims 1-10, **characterized** in that it is in a dried state that is

30   capable of being rehydrated.

12.   The use of the microfluidic device according to any of claims 1-11 in analytical systems in which an assay comprising one or more of the steps:

35   (a)   sample preparation,

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(b)   assay reaction and

(c)   detection,

at least one and preferably more than two of said steps

being carried out within the microfluidic device.

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7   B01L3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched. (classification system followed by classification symbols)

IPC 7   B01L   B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	EP 1 076 239 A (STUDIENGESELLSCHAFT KOHLE MBH) 14 February 2001 (2001-02-14) abstract; claims 1,7-17; figure 6 column 1, line 1 -column 1, line 18 column 4, line 26 -column 4, line 49 column 5, line 48 -column 6, line 4 column 6, line 33 -column 6, line 37	1-3,7, 11,12
A		4-6,8-10

X	DE 197 53 847 A (ROCHE DIAGNOSTICS GMBH) 10 June 1999 (1999-06-10) abstract; figure 1 column 3, line 67 -column 4, line 60 column 9, line 50 -column 10, line 33	1-3,7, 11,12
Y		4
A		5,6,8-10

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[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

° Special categories of cited documents :

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'O' document referring to an oral disclosure, use, exhibition or other means

'P' document published prior to the international filing date but later than the priority date claimed

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'X' document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

'Y' document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

'&' document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 March 2001	23/03/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Runser, C

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99 58245 A (AMERSHAM PHARM BIOTECH AB ;ALLMER KLAS (SE); ANDERSSON PER (SE); L) 18 November 1999 (1999-11-18) cited in the application abstract; figures 6-8 page 2, line 7 -page 5, line 30 page 7, line 11 -page 8, line 25 page 10, line 8 -page 11, line 23	4
A		1-3,5-12
A	US 5 250 613 A (BERGSTROM KARIN ET AL) 5 October 1993 (1993-10-05) cited in the application the whole document	1-7,9,10
A	US 5 240 994 A (OSTERBERG EVA ET AL) 31 August 1993 (1993-08-31) cited in the application the whole document	1-7,9,10
A	US 5 858 188 A (SOANE DAVID S ET AL) 12 January 1999 (1999-01-12) cited in the application the whole document	1-12
A	EP 0 430 248 A (MOCHIDA PHARM CO LTD) 5 June 1991 (1991-06-05) abstract page 9, line 49 -page 10, line 12 page 10, line 33 -page 10, line 41	1,2,4,8, 11,12

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Int␣␣␣Jonal Application No

PCT/EP 00/12478

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
EP 1076239	A	14-02-2001	DE 19938002 A		15-02-2001
DE 19753847	A	10-06-1999	AU 2158399 A		28-06-1999
			CN 1284012 T		14-02-2001
			WO 9929429 A		17-06-1999
			EP 1035921 A		20-09-2000
WO 9958245	A	18-11-1999	AU 3624399 A		29-11-1999
			EP 1077771 A		28-02-2001
			GB 2350678 A		06-12-2000
			GB 2341924 A		29-03-2000
US 5250613	A	05-10-1993	SE 467308 B		29-06-1992
			AU 8747991 A		20-05-1992
			EP 0554318 A		11-08-1993
			JP 6502156 T		10-03-1994
			SE 9003363 A		23-04-1992
			WO 9207006 A		30-04-1992
US 5240994	A	31-08-1993	SE 467309 B		29-06-1992
			AU 8749491 A		20-05-1992
			EP 0554324 A		11-08-1993
			JP 6502201 T		10-03-1994
			SE 9003364 A		23-04-1992
			WO 9207023 A		30-04-1992
US 5858188	A	12-01-1999	US 5126022 A		30-06-1992
			AU 715268 B		20-01-2000
			AU 2436497 A		29-10-1997
			CA 2249886 A		16-10-1997
			EP 0990147 A		05-04-2000
			JP 2000508763 T		11-07-2000
			WO 9738300 A		16-10-1997
			US 6054034 A		25-04-2000
			US 5750015 A		12-05-1998
			US 6093296 A		25-07-2000
			AU 637895 B		10-06-1993
			AU 7467591 A		18-09-1991
			CA 2075969 A		29-08-1991
			EP 0521911 A		13-01-1993
			JP 3103031 B		23-10-2000
			JP 8327597 A		13-12-1996
			JP 2601595 B		16-04-1997
			JP 5504628 T		15-07-1993
			WO 9112904 A		05-09-1991
EP 0430248	A	05-06-1991	AU 642444 B		21-10-1993
			AU 6702690 A		06-06-1991
			CA 2031001 A		31-05-1991
			JP 3223674 A		02-10-1991
			US 5147607 A		15-09-1992